

AMENDMENTS TO THE SPECIFICATION

On page 7, replace the paragraph starting on line 20 with the following:

FIG. 1 depicts a composite nucleic acid sequence (SEQID No. 1) and conceptual translation (SEQID No. 2) of full-length *Pnck* cDNA. Nucleotide coordinates are shown on the left. Amino acid coordinates are shown in boldface type on the right. A shaded box indicates the kinase catalytic domain, and a hatched box denotes the putative regulatory region. The in-frame upstream termination codons in the 5'-UTR and the putative polyadenylation sequence in the 3'-UTR are underlined by thin and thick lines, respectively. The putative initiation codon is boxed, and an asterisk denotes the stop codon. Arrows underline the regions corresponding to the degenerate oligonucleotides used to clone *Bstk3* initially.

On page 11, replace the paragraph starting on line 26 with the following:

The invention provides the *Pnck* gene, which has been cloned and fully sequenced as described in the Examples below, and the full length coding sequence derived from cDNA is set forth in FIG. 1 and SEQID No:1. Sequence data have been deposited with the EMBL/GenBank® Data Libraries under Accession No. AF181984.

On p. 31, replace the paragraph starting on line 25 with the following:

Individual clones were sequenced using the Sequenase® version 2 dideoxy chain termination kit (U.S. Biochemical Corp., Cleveland, OH). Putative protein kinases were identified by the DFG (aspartate-phenylalanine-glycine) consensus located in catalytic subdomain VI. DNA sequence analysis was performed using MacVector® 3.5 (Oxford Molecular Group, Oxford, UK) and the NCBI BLAST server (Altshul *et al. J. Mol. Biol.*, 215:403-410 (1990)).

On p. 32, replace the paragraph starting on line 1 with the following:

RNA Preparation and Analysis. RNA was prepared by homogenization of snap-frozen tissue samples or tissue culture cells in guanidinium isothiocyanate supplemented with 7 ml/ml 2-mercaptoethanol, followed by ultra-centrifugation through cesium

chloride as previously described (Marquis *et al.*, 1995; Rajan *et al.*, *Dev. Biol.*, 184:385–401 (1997)). Poly(A)⁺ RNA was selected using oligo(dT) cellulose (Pharmacia, Piscataway, NJ), separated on a 1.0% agarose gel (Seakem[®] LE, BioWhittaker Molecular Applications, Rockland, ME), and passively transferred to a Gene Screen membrane (New England Nuclear, Boston, MA). Northern hybridization was performed as described using ³²P-labeled cDNA probes corresponding to catalytic subdomains VI–IX of each protein kinase that were generated by PCR amplification of cloned catalytic domain fragments (Marquis *et al.*, 1995). In all cases calculated transcript sizes were consistent with values reported in the literature.

On p. 34, replace the paragraph starting on line 25 with the following:

The full-length *Pnck* cDNA sequence corresponding to the clone with the longest 5'-UTR, U7, was deposited with the GenBank[®] database (Accession No. AF181984).

On p. 34, replace the paragraph starting on line 27 with the following:

Sequence Analysis. Sequence analysis, including prediction of open reading frames, calculation of predicted molecular weights, multiple sequence alignment, and phylogenetic analysis, was performed using MacVector[®] (Oxford Molecular Group, Oxford, UK), ClustalW (Thompson *et al.*, *Nucleic Acids Research*, 22:4673-4680, 1994), ClustalX (Thompson *et al.*, *Nucleic Acids Res.*, 24:4876-4882 (1997)), and DendroMaker 4.0 (Tadashi Imanishi, Center for Information Biology, National Institute of Genetics). Pairwise and multiple sequence alignments of kinase catalytic domains I–XI were performed using the ClustalW alignment program. Calculations were made using the BLOSUM series (Henikoff *et al.*, *Proc Natl Acad Sci U S A* 89:10915-10919 (1992)) with an open gap penalty of 10, an extended gap penalty of 0.05, and a delay divergent of 40%. Phylogenetic calculations with the same parameters were performed using the ClustalX multisequence alignment program.

On page 36, replace the paragraph starting on line 3 with the following:

Consistent with the lengths of the isolated *Pnck* cDNA clones, this analysis

revealed an mRNA transcript approximately 1.6 kb in length (FIG. 2A), set forth as SEQID NO:1 (nucleic acid) and SEQID NO:2 (amino acid), respectively. A single band was detected in genomic DNA from both mouse and rat, confirming that, under these conditions, this *Pnck*-specific 3'-UTR probe recognizes a single locus. Ribonuclease protection analysis was performed as described (Marquis *et al.*, 1995). Body-labeled antisense riboprobes were generated using linearized plasmids containing nucleotides 1321 to 1509 of *Pnck* and 1142 to 1241 of β -actin (GenBank® Accession No. X03672) using [α -³²P]UTP and the Promega *in vitro* transcription system with T7 polymerase. A β -actin antisense riboprobe was added to each reaction as an internal control. Probes were hybridized with RNA samples at 58°C overnight in 50% formamide/100 mM PIPES (pH 6.7) (Piperazine-N,N'-bis[2-ethanesulfonic acid];1,4-Piperazinediethane sulfonic acid). Hybridized samples were digested with RNase A and T1, purified, electrophoresed on a 6% denaturing polyacrylamide gel, and subjected to autoradiography.

On p. 40, replace the paragraph starting on line 30 with the following:

For Northern hybridization analysis, RNA was separated on a 1% LE agarose gel and passively transferred to a Gene Screen membrane (DuPont NEN). Hybridization was performed as described using a random primed, ³²P-labeled cDNA probe encompassing nucleotides 1355–1529 of *c-myc* (GenBank® accession no. X01023), nucleotides 589–1287 of *cytokeratin 18* (GenBank® accession No. M11686), or a 1.2-kb fragment containing the entire open reading frame of *cyclin D3* (Marquis *et al.*, 1995).

On p. 41, replace the paragraph starting on line 5 with the following:

RNase protection analysis was performed as described (Marquis *et al.*, 1995). Body-labeled antisense riboprobes were generated using [α -³²P]UTP and the Promega *in vitro* transcription system with T7 polymerase in combination with linearized plasmids containing nucleotides 1142–1241 of β -actin, nucleotides 911–1056 of *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase) loci (GenBank® accession No. M32599), nucleotides 1321–1509 of murine *Pnck* (GenBank® accession No. AF181984), or a

region of human *PNCK* corresponding to nucleotides 538–842 of murine *Pnck*.

Riboprobes were hybridized with RNA samples overnight at 58°C in 50% formamide/100 mM PIPES (pH 6.7).